WE CLAIM:

1. The process for detecting the presence or absence of a viral RNA in a sample which comprises

(i) utilizing the sample as a template for hybridization with a first primer for a target viral RNA sequence present in substantially all transcripts of a virus, said primer being present in excess and positioned for extension through said target sequence;

(ii) extending said first primer to provide a double stranded first extension product having an RNA template strand including said target sequence and a DNA primer extension strand complementary to said target sequence;

(iii) denaturing said first extension product to provide a mixture containing said first primer and said separate RNA template and DNA primer extension strands;

(iv) providing, in the step (iii) mixture a second primer for extension through said sequence in said DNA primer extension strand which is complementary to said target sequence;

(v) annealing said first and second primers respectively to said separated RNA template strands and DNA primer extension strands;

(vi) extending said annealed primers;

(vii) denaturing the extension products produced in step (vi);

(viii) subjecting the product of step (vii) to hybridization with a labelled oligonucleotide probe for said target viral sequence or for a sequence complementary to said target sequence.

2. A process as defined by claim 1 in which steps (v), (vi), and (vii) are repeated prior to step (viii).

3. A process as defined by claim 1 or claim 2 in which said second primer is present during steps (i), (ii), and (iii).

4. A process as defined by claims 1, 2 or 3 in which said viral RNA is HIV-1 RNA and in which said target viral RNA sequence is within the 3′ ORF or the 5′ LTR region of the HIV-1 RNA.

5. A process as defined by claim 4 in which

(i) said target viral sequence is the BamHI-Sst-I fragment within the 3′ ORF region of the HIV-1;

(ii) said first primer includes the sequence 5′TGAATTAGCCCTTCCAGTCC;

(iii) said second primer includes the sequence 5′ATGCTGATTGTGCCTGGCTA; and

(iv) said probe includes the sequence 5′AAGTGGCTAAGATCTACAGCTGCCT.

6. A process as defined by claim 4 in which said target viral sequence is within the 5′ LTR region of HIV-1.

7. A process as defined by claim 6 in which

(i) said first primer includes the sequence 5′TGAGTGCTTCAAGTAAGTGTGTGCCC 3′;

(ii) said second primer includes the sequence 5′GTCGCCGCCCCTCGCCTCTTGCCGT 3; and

(iii) said probe includes the sequence 5′CGAAAGGGAAACCAGAGCTCTCTCG 3′.

8. A process as defined by claims 1, 2, or 3 in which said viral RNA is human immunodeficiency virus (HIV) RNA or human cytomegalovirus (HCMV).

9. A process as defined by claims 1, 2 or 3 for detecting the presence or absence of viral RNA in a peripheral blood sample.

10. A process as defined by claims 1, 2, or 3 for detecting the presence or of HIV-1 or HCMV in a peripheral blood sample.

11. The process for detecting the presence or absence of a human cytomegalovirus (HCMV) in a sample which comprises:

(i) utilizing the sample as a template for hybridization with a first primer for a target viral RNA sequence present in all transcripts of said HCMV, said primer being present in excess and being positioned for extension through said target sequence;

(ii) extending said first primer to provide a double stranded first extension product having an RNA template strand including said target sequence and a DNA primer extension strand including a sequence complementary to said target sequence;

(iii) denaturing said first extension product to provide a mixture containing said first primer and separate RNA template and DNA primer extension strands;

(iv) providing in the step (iii) mixture a second primer for extension through said sequence in said DNA primer extension strand which is complementary to said target sequence;

(v) annealing said first and second primers respectively to said separated RNA template strands and DNA primer extension strands;

(vi) extending said annealed primers;

(vii) denaturing the extension products produced in step (vi);

(viii) adding a labelled oligonucleotide probe for said target viral sequence to the product of step (vii).

12. A process as defined by claim 11 in which steps (v), (vi) and (vii) are repeated prior to step (viii).

13. A process as defined by claim 11 in which said second primer is present during steps (i), (ii) and (iii).

14. A process as defined by claim 11 in which

(i) said first primer includes the sequence 5′ CGAGACACCCGTGACCAAGG 3′;

(ii) said second primer includes the sequence 3′ CTCTTTCTACAGGACCGTCT 5′; and

(iii) said probe includes the sequence 3′ AAGGACGTCTGATACAACTCCTT 5′.

15. A process as defined by claim 11 for detecting the presence or absence of RNA from a human cytomegalovirus late gene transcript in a sample in which said target viral sequence includes nucleotides from the coding sequence of p64, said first primer includes the sequence 5′ AAAGAGCCCGACGTCTACTACACGT 3′, said second primer includes the sequence 3′ CGCGTGCTCGACCAAACGAGGTACCTCTTG 5′, and said probe includes the sequence 3′ CGCGTGCTCGACCAAACGAGGTACCTCTTG 5′.

16. A process which comprises:

(i) providing an amplification reaction mixture of a viral RNA, including a target sequence, a primer for annealing to said target sequence, reverse transcriptase, and a second primer for the annealing to and for extending across the DNA complement of said RNA target sequence;

(ii) annealing said first primer to said target sequence;

(iii) extending said first primer across said target sequence to provide a first extension product having an RNA target sequence strand and a DNA primer extension strand;

(iv) denaturing said first extension product to provide separate RNA target sequence and DNA primer extension strands;

(v) annealing said first and second primers to said separate RNA target sequence strands and DNA primer extension strands;

(vi) extending said annealed primers to produce a second extension product;

(vii) denaturing said second extension product;

(viii) repeating steps (ii) through (vii);

(ix) subjecting the amplification product after step (viii)to hybridization with a labelled oligonucleotide probe for said target sequence or for a sequence complementary to said target sequence.

17. A process as defined by claim 1 or 2 in which

(a) at least one synthetic RNA sequence which does not include said target sequence of which includes substantially more or less nucleotides than said target sequence is subjected, with said sample to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify said target sequence and said reference sequence;

(b) the amplification products of step (a) are denatured and thereafter separately and sequentially subjected to hybridization conditions with oligonucleotide probes for said target sequence and said reference sequence.

18. A process for minimizing false negative data in the identification of a target viral RNA sequence in a peripheral blood cell sample which comprises:

(i) selecting said target viral RNA sequence;

(ii) simultaneously subjecting

(a) said sample and

(b) at least one synthetic RNA sequence which does not include said target sequence or which includes substantially more nucleotides than said target sequence

to polymerase chain reaction amplification under conditions appropriate to simultaneously amplify said target sequence if present in said sample and said reference sequence;

(iii) denaturing the amplification products produced by step (ii);

(iv) subjecting said denatured amplification products of step (iii) to hybridization conditions separately and sequentially with probes homologous to said target sequence and to said reference sequence,

each of said probes being removed from a sequence with which it is hybridized prior to the separate and sequential subjection of said amplification products to hybridization with another of said probes;

(v) determining whether said amplified target and reference sequences hybridized with said probes homologous therewith.

19. A process as defined by claim 18 in which the reference nucleotide sequence utilized in step (ii) is

(i) a sequence present in the T-cell receptor expressed by cells affected by the virus containing said viral RNA;

(ii) a preselected RNA sequence present in substantially all of the cells of said sample,

(iii) a sequence including said target and constructed by a multi-base insertion into a site in said viral RNA preselected with respect to said target sequence;

(iv) a beta actin sequence.

20. A process as defined by claim 18 in which said target viral sequence is located within the 3′ ORF region of HIV-1 and in which the reference sequence utilized in step (ii) is located in the constant region of the beta chain of the T-cell receptor expressed T-cells affected by HIV-1.

21. A process as defined by claim 20 in which the reference sequence utilized in step (ii) is a beta actin sequence.

22. A process as defined by claim 20 in which the reference sequence utilized in step (ii) is a sequence formed by inserting a multi-base pair sequence into the 3′ ORF region of HIV-1.

23. A process as defined by claim 18 or 19 in which at least one of the primers utilized in conducting the polymerase chain reaction in step (ii) includes the T-7 RNA polymerase sequence.

24. A process as defined by claim 18 in which said target viral sequence is in the HCMV major immediate early (IE) gene.

25. A process as defined by claim 18 in which said target viral sequence comprises RNA from the transcription of late HCMV genes.

26. A process as defined in claim 18 wherein a predetermined quantity of the reference sequence is utilized in step (ii)(b); the probes utilized in step (iv) are labelled; and, the presence or absence of the target sequence and the presence of the reference sequence in the denatured amplification products of step (iii) is detected in step (iv) by Southern blot hybridization with said labelled oligonucleotide probes.

27. A process as defined by claim 26 in which the relative quantification of the target sequence is determined by comparison with the amount of signal obtained from the hybridized target sequence with the amount of signal obtained from the hybridized predetermined quantity of the reference sequence.

28. The process as defined in claim 26 in which the probes utilized in step (iv) are labelled with an isotope or a fluorophore.

29. A process as defined by claim 19 in which at least three of said reference sequences (i), (ii), (iii) and (iv) are simultaneously subjected to amplification conditions with the sample in step (ii).

30. A process as defined by claim 26 in which the amount of signal obtained from the hybridized target sequence is compared with the amount of signal obtained from the hybridized predetermined quantity of the reference sequences.

add B2

Add E'

Add F'